A novel approach for the production of DODAB:MO lipoplexes: the influence of temperature on the physicochemical characteristics and cell transfection efficiency

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INTRODUCTION: Cationic liposomes/ DNA complexes (lipoplexes) have been widely used as nano-carriers for animal cell transfection, with the neutral lipid (*helper*) playing a determinant role for the efficiency of this process due to the formation of non-lammellar intermediates that are akin to membrane fusion process [1]. We have developed a novel formulation containing the cationic agent dioctadecyldimethylammonium bromide (DODAB) and 1-monooleoyl-rac-glycerol (MO) as helper lipid [2]. In previous studies, we have demonstrated a strong dependence of the DNA complexation rate with several structural parameters such as the monoolein content or the DNA/cationic lipid charge ratio (+/-) [3]. The preparation method itself influences the structural properties of the lipoplexes formed as well as their lipofection capacity. This study addressed the effect of preparation temperature (25ºC or 50ºC) on DODAB:MO lipoplexes (molar ratios 4:1 and 2:1) physicochemical properties, as well as on their cell transfection efficiency (TE).

METHODS: Fluorescence microscopy (FM), Dynamic Light Scattering (DLS) and Zeta Potential (ζ) were used to study the physicochemical properties of the lipoplexes (morphology, size and electrical charge). Transfection efficiency was evaluated *in vitro* cultured human cells (293T cell line) by measuring the activity of β-galactosidase, the reporter gene in the plasmid delivered to these cells.

RESULTS: The lipoplexes, at charge ratio (CR) (+/-) 4, prepared at 50ºC, were smaller and presented higher charge density (~ +30 mV for both molar ratios) when compared to lipoplexes at the same charge ratio (CR = +/-4) prepared at 25ºC with superficial charge densities, +15 mV and +18 mV for (2:1) and (4:1) molar rations, respectively, *(Fig.1)*. The lipoplexes formed at the referred DODAB:MO molar ratios may have different structures, varied from lamellar to other structures, due to the tendency of MO to form structures with negative curavature. Transfection efficiency was not significantly different for the tested conditions and formulations *(Fig.1).*

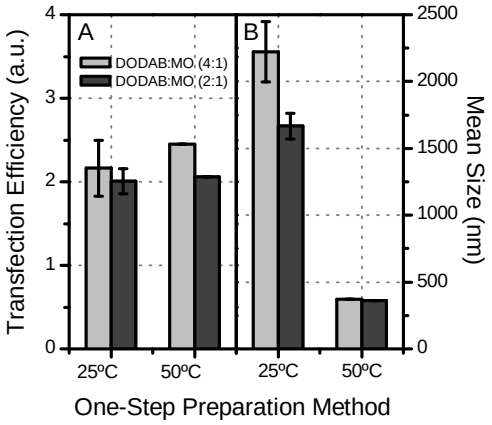

Fig.1: Lipoplex size at CR (+/-)4 and transfection efficiency.

DISCUSSION & CONCLUSIONS: These results indicate that the protocol of preparation is important for lipoplex physicochemical properties. At 50ºC the liposomes are in the liquid-crystalline phase implying a high mobility of the lipids, which leads to production of smaller lipoplexes with higher surface charge densities. At 25 ºC the DODAB:MO (4:1) is in the gel phase, and DODAB:MO (2:1) in the liquid-crystalline phase indicating that MO content influences lipoplexes structure in this preparation temperature. Nevertheless, it was not evident a linear correlation between lipoplex size, charge density and gene delivery efficiency, suggesting that other factors are influencing the transfection process, such as the route of lipoplex internalization, which is currently under study.

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